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1. Conner et al. (1983) PNAS 80: 278-282.

2. Rollini et al. PNAS (1985 Nov) 82(12): 7197-7201.

3.Gorski et al. IMMUNOGENTICS (1987) 25(6):379-402.

4.. de Preval et al. IMMUNOGEENTICS (1987) 26(4-5): 249-257.

5. Irle et al. J. EXPERIMENTAL MEDICINE (1988 Mar 1) 167(3): 853-872.

6. Andersson et al. IMMUNOGENETICS (1988) 28 (1): 1-5.

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The single DR_β gene of the *DRw8* haplotype is closely related to the $DR_\beta 3III$ gene encoding DRw52

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Abstract. In most individuals two *HLA-*DR_β genes are expressed from each chromosome. One of these genes encodes one of the classical DR specificities, while the other encodes either of the supertypic DRw52/DRw53 specificities. In addition to these genes usually one or two DR_β pseudogenes are present. In contrast, the *DRw8* chromosomal region only contains a single DR_β gene. To determine the relationship of this single gene to the multiple DR_β genes of other DR specificities, comparisons of Southern genomic blots were carried out. In this analysis genomic clones for each individual DR_β chain locus were included. The $DR_\beta w8$ gene was indistinguishable from the $DR_\beta III$ gene of DR3 cells (encoding DRw52), suggesting that it is closely related to the latter gene. The functional implications of this finding are discussed.

Introduction

Class II histocompatibility antigens in man are encoded by the *HLA-D* region. Serological, cellular, and, more recently, molecular cloning analyses have shown that this region is composed of three major subregions, *HLA-DR*, *-DQ*, and *-DP*. From each subregion at least one class II molecule consisting of an α and a β chain is expressed (Albert et al. 1985). The class II antigens are extremely polymorphic (Bach 1985). A large number of serologically defined DR specificities are known. By the use of cellular methods several of these specificities have been further split. These specificities correspond to allelic series of DR_β chains, while the DR_α chain is invariable. Two separate allelic series of DR specificities, the classical DR and DRw52/DRw53, have been established by serological studies. The DR series involves a large number of alleles, while the DRw52/DRw53 polymorphism is much more restricted (reviewed in Mach et al. 1986). The DRw52 specificity is carried by DR3, 5, w6, and w8 individuals, whereas those with DR specificities 4, 7, and w9 have the DRw53 specificity. More than one DR_β chain is expressed from each chromosome for most of the DR specificities (Rollini et al. 1985, Giles and Capra 1985). Epitopes corresponding to the supertypic specificities DRw52 and DRw53 are present on β chains separate from those displaying the classical DR specificities (Tanigaki and Tosi 1982).

In addition to the sequence variability between *DR* alleles there is a variability in the number of DR_β genes (Böhme et al. 1985). Molecular cloning and genomic hybridizations have shown that three DR_β genes, designated *I*, *II*, and *III*, are present in the *DR3*, *5*, and *w6* haplotypes (Rollini et al. 1985). $DR_\beta I$ encodes the DR3 specificity, $DR_\beta III$ corresponds to the DRw52 epitope (Gorski et al. 1985), while $DR_\beta II$ is a pseudogene. In the *DR4* haplotype four DR_β genes exist, two of which are pseudogenes, while the other two encode the DR4 and DRw53 specificities. Hybridization experiments have shown that the *DR7* and *DRw9* haplotypes have a gene organization similar to *DR4* (Böhme et al. 1985, Andersson et al. 1987). These studies also indicated that the *DR2* haplotype contains three and the *DR1* haplotype two DR_β genes. In the *DRw8* haplotype a single DR_β gene has been found (Böhme et al. 1985). The present study was undertaken to clarify the relationship of this single gene to the multiple genes encountered in the *DR3* and *DR4* haplotypes. Since detailed molecular information is available from the cloned DR_β genes of the *DR* haplotypes *DR3* and *DR4* it is possible to assign every single restriction fragment hybridizing with a DR_β probe in Southern hybridizations of restricted DNA of these haplotypes to a specific gene. To minimize allelic restriction site polymorphism which might make such a comparison impossible, we decided to use hybridization probes corresponding

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to the reasonably wellconserved 3′ portions of the DR_β genes.

Materials and methods

DNA sources. DNA was prepared from the following homozygous typing cells that were selected from the typing panels used at the State Institute for Blood Group Serology, University Hospital, Linköping, Sweden: PH, DR1/1; IML, DR2/2; DL, DR3/3; SA, DR5/5; and IH, DR7/7.

Cell lines SPL, DRw8/w8 and KOZ, DRw9/DRw9 were obtained from Dr. J. Bodmer of the Tissue Antigen Laboratory, Imperial Cancer Research Fund Laboratories, London, England. Additional DNA was prepared from the cells used in our earlier work: BO, DR4/4 (Andersson et al. 1987) and GP, DRw6/w6 (Böhme et al. 1985).

Hybridization probes. Two DR_β-specific probes corresponding to (i) the cytoplasmic exon with flanking introns and (ii) the DR_β 3′-untranslated exon were used (Table 1). After restriction enzyme digestions of the clones, the probe fragments were isolated by preparative agarose gel electrophoresis. Fragments were radioactively labeled to high specific activity with $\alpha(-^{32}P)$ deoxycytidine triphosphate by either nick-translation (Rigby et al. 1977) or random priming (Feinberg and Vogelstein 1984).

DNA preparation and Southern blot analysis. DNA was isolated from either 5 ml of peripheral blood according to the method of Böhme and co-workers (1983) or 500 µl of pelleted cultured cells as described by Grosveld and co-workers (1982). Samples of 10 µg of human DNA were digested to completion with restriction enzymes according to the manufacturer's recommendations. The digests were separated by agarose gel electrophoresis in 0.7 and 1.5% gels, in 1 × TBE (89 m*M* Tris-borate buffer, pH 8.3, 2.5 m*M* ethylenediaminetetraacetate). Southern transfer of restricted DNA to Biodyne hybridization membranes (Pall) was made overnight with 1 × SSC (0.015 *M* sodium citrate buffer, pH 7.0, 0.15 *M* NaCl). Hybridizations were carried out in 40% formamide at 42 °C. Washings were performed at high stringency (0.2 × SSC, 0.25% sodium dodecyl sulfate) at 58 °C for 1 h.

Results and Discussion

To define the relationship of the DR_β gene carried by DRw8 individuals to those of the *DR3* and *DR4* haplotypes, genomic DNA samples from homozygous typing cells of DR specificities 1 to w9 were separately digested with Pst I, Bam HI, and Hind III and hybridized to a probe corresponding to exon 5 of DR_β genes (Table 1). Hybridization to Hind III-digested DNA is shown in Figure 1. In all cases a single hybridizing fragment was found for the DRw8 cells, which is in agreement with the proposal that this haplotype contains only a single DR_β gene. A single DR_β fragment in DRw8 cells was also obtained when genomic DNA digested with Pvu II was

Table 1. Documentation of hybridization probes

Probe corresponding to	Type of clone	Designation of clone	Restriction sites employed	Size of probe fragment (bp)	Reference
DR_β exon 5 and intron	Genomic	p801-2	Bgl II	376	Larhammar et al. 1985
DR_β exon 6	cDNA	pII-β-4	Pst I	220	Gustafsson et al. 1984

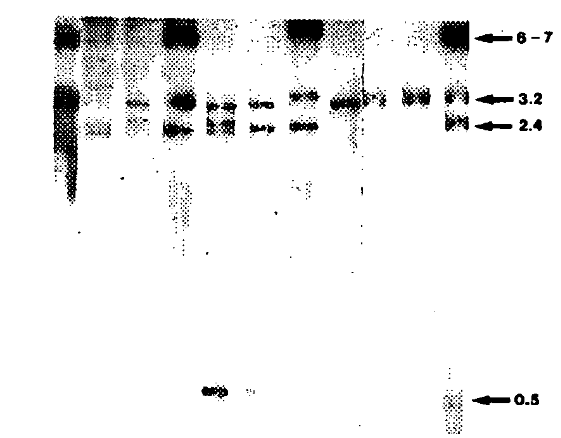

Fig. 1. Hybridization with the DR_β exon 5-specific probe to Hind III-restricted human genomic DNA corresponding to DR specificities 1 to w9. The sizes of restriction fragments are given in kilobases

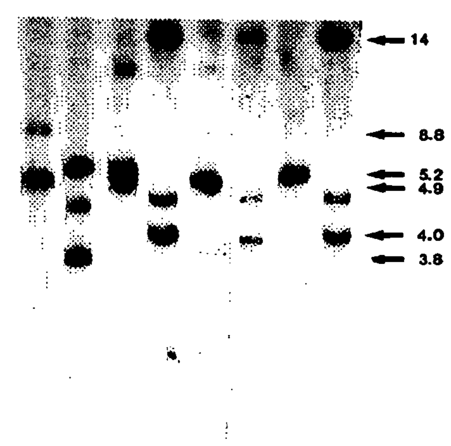

Fig. 2. Restriction fragment patterns of human genomic DNA, representing the same individuals as in Figure 1, digested with Pvu II and hybridized with the DR_β 3′-untranslated probe. The sizes of restriction fragments are given in kilobases

hybridized with the probe corresponding to the 3′-untranslated exon (Fig. 2). In all these experiments the hybridizing fragment of DRw8 cells coincided with fragments present in DR3, 5, and w6 DNA, but not with fragments in DR4, 7, and w9 DNA (Table 2). These findings suggested that the $DR_\beta w8$ gene is closely related to one of the genes present in the *DR3, DR5,* and *DRw6* haplotypes.

To further investigate this possibility, cosmid clones 4-1, 6-2, and 10-4, containing the $DR_\beta 3$ genes *I, II,* and *III*, respectively (Rollini et al. 1985), were run in parallel with DR3 and DRw8 genomic DNA on agarose gels after separate digestion with Pst I, Pvu II, and Hind III. The digests were hybridized with the DR_β probes used above, and the results are compiled in Table 2. A representative result obtained after Hind III digestion is shown in Figure 3. It is evident that all hybridizing fragments in the DR3 DNA can be accounted for by fragments in the three cosmids (Table 2 and Fig. 3, lanes 1–3). The DRw8 fragments coincided in all cases with fragments derived from the $DR_\beta 3III$ gene, i. e., the gene encoding the $DR_\beta w52$ molecule.

Table 2. Occurrence of bands in DNA from homozygous typing cells of different DR specificities digested with Bam HI, Hind III, Pst I, and Pvu II and hybridized with the DR_β probes from Table 1

Probe and enzyme	Size*	DR specificity									Restriction fragments corresponding to $DR_\beta 3$ genes*
		1	2	3	4	5	w6	7	w8	w9	
DR_β 3′UT, Pvu II	3.8		+				ND†				
	4.0				+			+		+	
	4.5		+								
	4.6				+			+		+	
	4.9	+		+		+					βI
	5.2			+					+		βIII
	5.3		+								
	6.8	+									
	11			+		+					βII
	14				+			+		+	
DR_β exon 5 and intron, Pst I	0.1				+			+		+	
	0.45	+	+								
	0.52		+	+	+	+	+	+		+	βII
	0.57				+			+		+	
	0.65	+									
	0.9		+								
	0.95			+		+	+		+		βIII
	1.0			+		+	+				βI
	1.1				+			+		+	
	2.0				+			+		+	
DR_β exon 5 and intron, Bam HI	0.3	ND†		+	+	+	+	+		+	ND
	0.6				+			+		+	
	0.85		+	+		+	+		+		
	10				+			+		+	
	14		+	+		+	+				
DR_β exon 5 and intron, Hind III	0.5			+		+	+				βI
	2.2		+								
	2.3			+		+	+				βI
	2.4		+		+			+		+	
	2.5			+		+					βII
	3.0	+		+		+	+		+		βIII
	3.2				+			+		+	
	3.6		+								
	6.0	+									
	6.5				+			+		+	
	7.5				+			+		+	

* Sizes in kilobases
† ND, not done
* The restriction fragments corresponding to different DR_β genes were determined by Southern blot analysis of representative cosmid clones (see text)

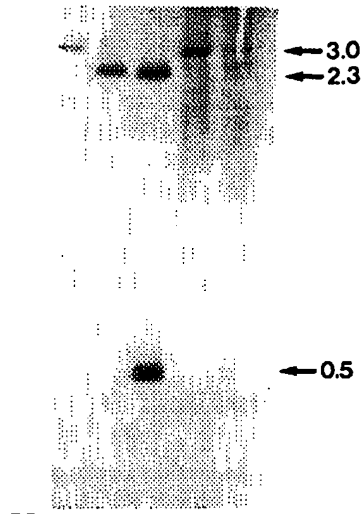

Fig. 3. Comparison of DR3 and DRw8 genomic DNA with cosmid clones $DR_{\beta}3I$ (4-1), $DR_{\beta}3II$ (6-2), and $DR_{\beta}3III$ (10-4), digested with Hind III and hybridized with the DR_{β} exon 5 probe

This result clearly indicates that the *DR*$_{\beta}$*w8* gene is closely related to the *DR*$_{\beta}$*w52* gene and thus might be a *DRw52* allele. In this respect, it is of interest that oligonucleotide probes specific for each of the three *DR*$_{\beta}$*III* alleles identified by DNA sequencing (*DRw52a, w52b*, and *w52c*) are negative in hybridization with DRw8 DNA, suggesting the existence of an additional *DR*$_{\beta}$*III* allele (Tiercy et al. 1987). Interestingly, DRw8 cells have been reported to react with some but not all DRw52 sera (Baldwin et al. 1985). This fact and the results above suggest that the DRw8 and DRw52 epitopes are carried by the same DR_{β} chain. Serologically, DRw52 and DRw53 are regarded as belonging to the same allelic series (Bach 1985). This notion was recently questioned, since a comparison of the restriction maps and DNA sequences of the *DR*$_{\beta}$*w53* gene with those of the three *DR*$_{\beta}$*3* genes *I, II,* and *III* suggested that the *DR*$_{\beta}$*w53* gene is more closely related to *DR*$_{\beta}$*3II* than to *DR*$_{\beta}$*3III* encoding the DR_{β}w52 chain (Gorski et al. 1987). It should be noted, however, that careful sequence analyses of the four *DR*$_{\beta}$*4* genes showed that extensive exchanges of sequences have occurred among these nonallelic genes, making allelic relationships difficult to establish (Andersson et al. 1987). The sequence of the DR_{β}w8 first domain exon was recently reported by Bell and co-workers (1987). A comparison of this sequence with those of other DR_{β} first domain exons does not provide any clear answer concerning the relationship of the *DR*$_{\beta}$*w8* gene. More extensive sequence information is needed to clarify this matter.

The results of the present study suggest that the single DR_{β} gene in the *DRw8* haplotype is not a *DR*$_{\beta}$*I* gene but more likely a *DR*$_{\beta}$*III* gene (following the nomenclature for DR_{β} genes in the *DR3* haplotype). This finding is surprising and has interesting functional implications. Previous studies have indicated that the *DR*$_{\beta}$*I* gene should be the immunologically most important gene in haplotypes which express more than one DR_{β} molecule. It has been reported that the major DR_{β} polymorphism revealed by serological (DR) or cellular (Dw) methods is encoded by the *DR*$_{\beta}$*I* gene and not by the *DR*$_{\beta}$*III* gene (Gorski et al. 1987). Further, only a minority of all DR-restricted antigen-specific T-cell clones are indeed restricted by allelic products of *DR*$_{\beta}$*III* (C. Irlé, personal communication). Finally, DRw52 and DRw53 molecules have been shown to be expressed at a four- to tenfold lower level than the classical DR molecules (Gregersen et al. 1986, Kratzin et al. 1986, Berdoz et al. 1987). Whether this also is the case for the DRw8/DRw52 molecule is not known. If so, it can be suggested that DR-restricted antigen presentation might be less efficient in DRw8 cells, since they express only a *DR*$_{\beta}$*III*-related gene. Furthermore, it is well established that the repertoire of class II molecules on antigen-presenting cells influences the immune response (Benacerraf and Germain 1978, Zinkernagel and Doherty 1979), and it has been a common view that a broad class II repertoire is beneficial for the individual. If this latter assumption is correct, it would imply that individuals homozygous for *DRw8*, who only express a single variant of the DR_{β} chain, should be at a disadvantage compared with individuals of other known *DR* genotypes who express two to four different variants of DR_{β} chains. To the best of our knowledge there is no data indicating that this should be the case. However, *DRw8* homozygotes must be very uncommon because of the fairly low haplotype frequency of *DRw8*.

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